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Autonomous Immunity in Mucosal Epithelial Cells: Fortifying the Barrier against Infection

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Abstract

Mucosal epithelial cells express an autonomous innate immune response that controls the overgrowth of invaded bacteria, mitigates the harmful effects of the bacteria carried within, and does not rely on other external arms of the immune response. Epithelial cell autonomous innate immunity “respects” the social biology of invading bacteria to achieve symbiosis, and is the primary protective mechanism against pathogens.

Keywords

Innate immunity; mucosal epithelium; antimicrobial proteins; cell signaling; infection

1. Introduction

“The power which resides in him is new in nature, and none but he knows what that is which he can do, nor does he know until he has tried.”

— Ralph Waldo Emerson, *Self-Reliance*

The social lives of bacteria demand that they colonize mucosal surfaces in humans and animals. Bacteria can reside inside or outside of mucosal epithelial cells as they attempt to find adequate nutrition to survive and multiply. In general, bacteria do not seek to kill their hosts; death of the host eliminates the ability of human pathogens to survive too. Mucosal symbionts survive on and in mucosal epithelial cells but are controlled by innate and adaptive immune responses that target the mucosal surface and the interior of mucosal epithelial cells. Indeed, there is no adaptive immune response to many symbionts. By invading mucosal epithelial cells, symbionts and some pathobionts become largely protected against the adaptive immune response and innate immune factors found in the secretions that bathe the mucosal surfaces, including lysozyme, lactoferrin and lactoperoxidase. As we come to appreciate that the interior of mucosal epithelial cells contains communities of

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Conflict of interest

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infectious bacteria, we raise the question of how the interior of the cell is protected and why we do not die of an ever-growing ulcer or abscess. Furthermore, significant gastrointestinal pathogens such as *Listeria* and *Salmonella* first encounter the oral mucosa while contaminated food is being chewed and swallowed; yet infection of the oral mucosa does not occur. The oral mucosa is a formidable barrier against infection by pathological bacteria such as *Listeria* and *Salmonella*, yeasts including *Candida albicans*, and viruses such as HIV-1. The gastrointestinal mucosa is somewhat more permissive to infection by “true” pathogens like *Listeria* and *Salmonella*, responding with antimicrobial proteins produced by the intestinal epithelial cells and mucous from goblet cells [1]. Intestinal barrier function is enhanced when the epithelium senses metabolites such as taurine from the microbiota, activating the inflammasome and increasing production of IL-18 [2, 3]. IL-18 signals in an autocrine manner to increase production of antimicrobial proteins, maintain diversity of the microbiome, and prevent dysbiosis [2, 3]. When other microbiome metabolites such as spermine are sensed, the epithelium ceases production of antimicrobial proteins, IL-18 autocrine signaling prevents development of goblet cells, and a dysbiotic, disease-causing microbiome emerges [2, 3]. This contemporary example of cell autonomous epithelial immunity may serve as a paradigm for studies of other epithelial tissues.

This review will focus largely on the autonomous mechanisms of the squamous epithelia that line the oral cavity, oropharynx and the genitourinary tracts enabling resistance to infection by pathogens. Autonomous mechanisms function without reliance on the innate and adaptive immune mechanisms provided by white blood cells [4–6]. Cell autonomous immunity is a widely expressed system of self-defense that functions in many cell types and appears to be protective against a wide range of pathogens. For the host to defend against durable pathogens such as *Mycobacterium tuberculosis*, several defensive strategies are required [7]. Some of these mechanisms reflect the co-evolution of the pathogen and the host and examples exist in virtually all phyla. Indeed, bacteria protect against infection by bacteriophage with an innate adaptive mechanism, the clustered regularly interspaced short palindromic repeats (CRISPR) system, which recognizes and excises foreign DNA in a sequence-specific manner [8]. Bacteria also defend nutritional territory by production of toxic peroxides [9, 10] and bacteriocins [11].

2. The mucosal epithelial barrier

In epithelia, the innate autonomous immune capacity of each cell contributes collectively to the barrier function of the tissue. Protecting the connective tissues from environmental insults including pathogens, the barrier function is both biological and physical [12]. The physical barrier is constructed by intercellular attachment structures that bond each epithelial cell to one another and to the basement membrane. Apically, tight junctions loosely stitch the cells to one another to form a semi-permeable barrier. Formed by cadherin adhesive proteins ringing the apical aspect of each cell, adherens junctions also bind cells together. Extending across the plasma membrane to the cytoplasmic face of the cells, adherens junctions also connect to the actin-based cytoskeleton. A third structure that attaches neighboring epithelial cells together is the desmosome, which appear to be rigid plaques that create strong “spot-welds” between cells. In membrane regions lacking attachment structures, epithelial cells are separated by a gap of about 30 nm. To keep the epithelial

syncytia tightly attached to and covering the underlying connective tissue, hemidesmosomes cement the basal aspect of the cells to the basement membrane. These several attachment structures enable the epithelium to resist abrasion during typical functional activity like mastication and speech, respiratory air movement during exercise or coughing, gastrointestinal peristalsis, and genitourinary activity.

Gap junctions also bridge mucosal epithelial cells. Formed by complexes of proteins of the connexin family, these proteins organize into transmembrane gated-hemichannels. When hemichannels align between adjacent cells, a gap junction forms. The gap junction facilitates intercellular communication mediated by transfer of small molecules from one cell to its neighbor. Similarly, unaligned hemichannels can release biologically active small molecules to be bound and signal via proximal cell receptors. Bacteria and other microbes can very rapidly stimulate release of stored small molecules from hemichannels and gap junctions. To the extent that such signaling contributes to innate defense of the epithelium, this signaling mechanism may be the most rapid of any that are known to contribute to innate autonomous immunity.

Mucosal epithelial tissues in different anatomic sites show many similar activities and responses but can also differ markedly depending on the anatomic site. Indeed, the mucosal epithelia in various anatomic sites contains epithelial cells of either of two morphologies: squamous and cuboidal/columnar. In some locations, cells between mucosal tissues show transitional morphologies and some variations in size and shape.

2.1 Squamous epithelium

In the oral cavity, oropharynx and genitourinary tissues, the mucosal epithelium is an interdigitating stack of squamous cells, developed from basal cells, including stem cells, at the basement membrane. As the basal cells differentiate, new cells emanate from the basal layer to the mucosal surface in regions of increasing maturity. As they mature and migrate to the surface of the epithelium, squamous cells lose the ability to synthesize protein and divide, eventually to be sloughed. Between the squamous epithelial cells are sandwiched intraepithelial lymphoid cells in low abundance including Langerhans cells, $\gamma\delta$ T cells and intraepithelial lymphocytes. These cells appear to provide immune surveillance of the mucosal surface and facilitate regional or central processing of antigen. These lymphoid cells, however, are unlikely to function directly as innate immune effectors in the control of mucosal commensals and pathogens.

2.2 Columnar/cuboidal epithelium

The pulmonary and gastrointestinal mucosae form from a single layer of epithelial cells with morphologies ranging in shape from columnar to cuboidal. The single cell thick epithelium contrasts to the stratified squamous mucosal epithelia. In both epithelia, cells connect to one another and the basement membrane similarly, recapitulating the same attachment organelles and functions. In the unicellular layer of pulmonary and gastrointestinal (GI) epithelia, intraepithelial lymphoid cells are more sparse than in squamous mucosal epithelium. The GI epithelial layer, however, contains M cells, which can recognize and translocate antigens and bacteria from the lumen to be processed by the gut-associated lymphoid tissue (GALT) on

the serosal side of the epithelium [13]. Also residing in crypts within the epithelial layer, Paneth cells are a copious source of antibacterial peptides. Goblet cells are modified epithelial cells that release mucus to enhance the barrier on the luminal side of the epithelium. Although residing in the subepithelial mucosa, recently described innate lymphoid cells cooperate with the innate immune function of the GI and pulmonary epithelia [14]. Relying on cytokine signals from epithelial and dendritic cells in response to mucosal pathogens, the innate lymphoid cells release effector molecules that control growth of extracellular and intracellular microbial pathogens. Intraepithelial lymphocytes (IELs), in contrast, reside in the paracellular space between epithelial cells or are associated with the gut-associated lymphoid tissues [15]. In health, there are fewer than 25 IELs per 100 epithelial cells. Of these heterogeneous, antigen-experienced T cells, up to 60% are $\text{TCR}\gamma\delta^+$ cells and do not require priming. Upon encounters with antigens, the IELs release cytokines that maintain quiescence and ensure the integrity of the intestinal epithelium.

We will now focus on what mucosal epithelial cells do to resist or combat infection, without the cooperation or influence of neighboring white blood cells.

3. The biology of epithelial infection

During functional activities, the luminal (apical) epithelial surface encounters microbes arising from the external environment, trafficking from other mucosal compartments, and residents of the specific mucosal tissue. Many species invade the epithelial cells to seek refuge from antimicrobial substances in the mucosal secretions and prevent clearance by swallowing or other movement of the viscous mucin-rich mucosal fluids culminating in excretion. Since complex viscous secretions coat the mucosal epithelium, microbes are challenged to interact directly with the epithelium. To traverse the coating film and bind to epithelial cells, the microbes must often digest the complex mucin-rich secretory fluid. Once the mucous moat has been crossed, microbes bind the epithelial cells using surface appendages such as pili, fibrils, adhesive proteins and lipoproteins (adhesins), and phospholipid polymers. These microbial structures may also represent pathogen-associated molecular patterns (PAMPs), which can signal a cell autonomous immune response by engaging surface Toll-like receptors (TLRs) and protease-activated receptors (PARs), cytoplasmic receptors such as nucleotide-binding and oligomerization domain (NODs), and retinoic acid inducible gene (RIGs) (see below).

Epithelial innate immunity can be augmented by communication through gap junctions between infected and uninfected cells. In airway epithelial cells, engagement of TLR2 by *Pseudomonas aeruginosa* causes an immediate Ca^{2+} flux that spreads to adjacent cells through via gap junction communication [16]. The Ca^{2+} flux stimulates proinflammatory NF- κ B and MAPK signaling. Blocking the functions of gap junctions reduces the chemokine response of cells, indicating that innate immune signals can spread from cell-to-cell through open gap junctions. *P. aeruginosa* engagement of TLR2 also induces tyrosine phosphorylation of connexin43 (Cx43), a key protein component of the gap junction hemichannel. By 4 h after stimulation by *P. aeruginosa*, phosphorylation signaling activates c-Src decreasing gap junction communication. Regulating the opening and closing of gap

junctions (gating) may enable control of the strength of the inflammatory response in epithelial tissues.

In the gut, invasion by *S. flexneri* activated Nod 1 in the intestinal epithelial cells [17]. Within 30 minutes of infection, the adjacent cell responded by activating NF- κ B, JNK, ERK, and p38 signaling, which induce IL-8 secretion. Activation of the bystander cell occurred through gap junction communication. To restrict cell antimicrobial responses, invading pathogens often come equipped with virulence factors that suppress cell activation. *S. flexneri* injects OspF, an inhibitor of JNK, ERK and p38 signaling, into the cytosol of the infected cell. Using cell-cell communication through gap junctions, the infected cell bypasses the effects of inhibitor proteins by upregulating cytokine secretion in neighboring cells. By co-opting uninfected cells through gap junction communication, the epithelium functions as a collective barrier to produce robust defense.

During invasion of host cells, however, Shigella can also stimulate the opening of connexin 26 hemichannels, allowing ATP to be released into the medium. The rise in extracellular (e)ATP allows for greater Shigella invasion and cell-to-cell spread [18]. *Yersinia enterocolitica* also exploits connexin hemichannels for invasion [19]. *Y. enterocolitica* increases expression of Cx43 in HeLa cells and bacterial internalization. Hence, connexin hemichannels can be both exploited by pathogens and involved in the innate immune protection of the epithelia.

4. Pathogen sensing and restriction

Epithelial cells are equipped to sense or recognize microbes or their characteristic PAMPs. At the plasma membrane, the Toll-like receptors (TLRs) are a family of signaling receptors for PAMPs. Receptors that recognize and respond to PAMPs are also termed, pathogen-recognition receptors (PRRs). Upon engaging certain PAMPs, some TLRs will traffic from the plasma membrane into the endoplasmic reticulum and endosomes. Able to engage PAMPs that escape endocytosis, the cytoplasm presents PRRs including NOD 1 and 2, RIG-1, and the melanoma differentiation associated gene-5 (MDA5) [20, 21]. Hence, the architectural features of the cell include specialized antimicrobial mechanisms, each presumably designed to sense the microbe and fragments of microbes (PAMPs) before and after invasion, localized to survey the external environment and the cell interior [22]. Each type of PRR signals and activates an epithelial transcriptional response through pathways involving NF- κ B, mitogen activated protein (MAP) kinases and interferon regulatory factors (IRFs). Although many epithelial cytokines are proinflammatory and invite involvement of inflammatory cells, the cell autonomous immune response can involve autocrine signaling by certain released cytokines. As we reported [23], IL-1 α released from epithelial cells in response to certain bacteria, for example, engages the IL-1 receptor (IL-1R) on the same or proximal epithelial cells (Figure 1). Signaling through the IL-1R augments transcription of cell protective antimicrobial proteins, such as calprotectin (S100A8/A9) without support from inflammatory or immune cells. Another functional outcome of pathogen sensing is the formation of autophagosomes [24, 25]. Stimulated by engagement of TLRs, the IL-1R, or cytoplasmic NOD signaling, autophagosomes restrict microbial invasion and translocation through the mucosal epithelium. Similarly, TLR engagement on mucosal epithelial cells, for

example, also increases expression of antimicrobial peptides (AMPs). Production of AMPs and formation of autophagosomes are two effector mechanisms of cell autonomous immunity that characterize mucosal epithelial cells.

4.1 Toll-like Receptors

To respond to PAMPs, TLR cellular sensors traffic from the surface to the interior of the cell. Hence, the TLRs are compartmentalized, such that TLR1 to 4 are presented on the cell surface whereas TLR5 to 9 are associated with the endosomes [22]. TLR2 can associate with TLR1 or 6, increasing the specificities of PAMPs that can be engaged and also contribute to trafficking of the complexes to the endosomes. Since many pathogens internalize into the endosomes, engagement of intracellular TLRs can orchestrate an innate immune response. For example, TLR2 internalization (reflecting down-regulation from the surface) can trigger IFN production in monocytes/macrophages [26, 27] and in pulmonary epithelial cells by cooperation with endosomal TLR7 and 8 and MDA-5 [28]. Recently, HSV-2 infection of immature epidermal Langerhans cells was found to produce TNF and low levels of IFN β , whereas epidermal keratinocytes were unresponsive [29]. Whether squamous mucosal epithelial cells rely on TLR signaling for IFN production and innate antiviral activity has not yet been reported.

Differences in expression of TLRs may exist depending on the lineage and environmental challenges of the mucosal epithelial cells. Oral and gingival epithelial cells in vitro and in vivo express both TLR2 and 4 on the plasma membrane [30–32] [33]. Similarly, squamous tonsillar epithelium expresses TLR2 and 4 [34]. Expression levels of TLRs are differentially regulated in the presence of cigarette smoke [35], lipopolysaccharides common to the keystone pathogen in periodontal disease, *Porphyromonas gingivalis* [36, 37, 31, 30], and in the mucosal white lesion, lichen planus [38]. For example, the O-antigen of *P. gingivalis* caused increased TLR4 expression, which correlated with bacterial invasion, decreased apoptosis, and increased host cell survival of host epithelial cells [39]. *P. gingivalis* also increases the cell resistance to oxidative stress by activating FOXO1 [40]. Apoptosis and cell desquamation promote renewal of the mucosal epithelial barrier and dispose of cells with internalized microbes. By antagonizing these mechanisms, *P. gingivalis* appears to enable its own persistence.

Cervicovaginal (e.g., normal human vagina, ectocervix, and endocervix) squamous and transitional epithelial cells differentially express mRNA for TLR 1, 2, 3, and 5 to 9 [41, 42]. Endocervical and vaginal epithelial cells express TLR4. In the absence of TLR4, epithelial cells respond to Gram-negative bacteria and lysates but are unresponsive, for example, to lipooligosaccharide from *Neisseria gonorrhoeae* and LPS from *Escherichia coli*. Immortalized vaginal epithelial cells in 3D cultures express TLR2, TLR2/6, TLR3, and TLR5 based on responses to specific agonists [43]. The presence of commensal vaginal bacteria suppresses the proinflammatory TLR agonist-specific responses; pathogenic Staphylococci stimulate an inflammatory response. Whereas TLR2 and 4 signaling induce expression of antimicrobial peptides in vaginal epithelial cells [44], cervical epithelial cells upregulate hCAP18/LL-37 (CAMP) in vivo and in vitro in a TLR– independent manner [45] suggesting that several cell autonomous immune pathways may function in parallel.

4.2 HIV-1 encounters with mucosal epithelial cells and protease-activated receptors

The oral mucosal epithelium expresses HIV-1 co-receptors and alternate receptors as modeled on the human tonsils from children and adults [46]. The barrier function of oral epithelial cells is compromised by exposure to gingipain-expressing *P. gingivalis* [47]. As studied using multilayer, organotypic cultures of gingival epithelial cells, *P. gingivalis* arg- and lys-proteases (gingipains) contribute to increased FOXO1 and FOXO3 expression. In a FOXO1 and FOXO3- dependent manner, expression of genes involved with epithelial differentiation and apoptosis increase, while proinflammatory (TLR2 and TLR4) and barrier function genes (integrins) are reduced. The gingipains signal through protease-activated receptors (PARs) on the epithelial cells to selectively up-regulate the HIV-1 co-receptor CCR5 on oral mucosal keratinocytes [48–50]. Ordinarily, R5-tropic HIV-1 do not internalize into adult mucosal epithelial cells. Upon PAR activation and up-regulation of functional CCR5, infectious R5-tropic HIV-1 internalizes from the culture medium into mucosal epithelial cells [48]. Once internalized, the infectious HIV-1 can be transferred to proximal permissive cells. In adult oral epithelial cells, the R5-tropic virus is harbored but does not replicate and remains infectious for at least two days [51]. When promiscuous VSV-coated HIV-1 pseudovirus is used to infect the epithelial cells, replication occurs as evidenced by use of a GFP reporter system. Internalization of the virus by mechanisms other than CCR5-mediated events appears to be sufficient to bypass the HIV-1 restriction preventing replication. Although human saliva has been thought to inhibit HIV-1 replication during oral infection, the rate of uptake of infectious virus in the presence of saliva is more rapid than the rate of inactivation [52]. Hence, activation of PARs by certain mucosal pathogens can compromise epithelial cell autonomous immunity against HIV-1.

4.3 APOBECs

Epithelial restrictions against replication of HIV-1 [53] and other dsRNA-containing viruses include the APOBEC family of single-stranded DNA cytosine deaminases [54]. Epithelial cells express APOBEC3A (A3A), A3B and A3H. A3B appears to contribute to over-editing of HPV and signature mutations are found in head and neck and cervical carcinomas. APOBEC3 family members can inhibit integration through several mechanisms [55]. Furthermore, APOBEC3G induces sublethal mutagenesis of HIV-1 leading to viral variation [56]. Although the HIV-1 protein Vif can neutralize APOBEC3G, signature C-deamination mutations in patient samples indicate that escape occurs [57, 58]. HIV-1 virions can breach human and macaque penile epithelia atraumatically [59]. Yet, HIV-1 is unable to productively replicate in human adult epithelial cells (Herzberg and others).

4.4 Other anti-viral mechanisms

Tetherin restricts HIV-1 and other viruses at the cell surface. Tetherin complexes with the mitochondrion-associated autophagy suppressor LRPPRC [60]. By binding the suppressor, autophagy and mitophagy are enabled which may inactivate infectious virus. To HIV-1 infectivity is also controlled by human TRIM 37, which interferes with viral DNA synthesis [61]. Although fetal and neonatal mucosal epithelial cells are capable of productive infection by HIV-1 [62], as cells mature hBD2 and 3 are produced. Consequently, hBD2 and 3 tend to

inactivate HIV-1 that migrates into endosomal compartments of adult tonsil epithelial cells [63].

Once internalized into infected cells, HIV-1 or DNA viruses are sensed by intracellular sensors such as cyclic guanosine monophosphate (GMP)–adenosine monophosphate (AMP) synthase (cGAS) [64, 65]. In response, the cell produces the second messenger 2'3'-cyclic GMP-AMP (cGAMP), which activates antiviral mechanisms. During infection, viral particles can package cGAS-synthesized cGAMP (in addition to APOBECs as above), which can activate antiviral pathways in proximal infected cells. cGAMP can also be transferred by diffusion from cell-to-cell via gap junctions [66, 67]. When transferred through gap junctions or during uptake of virions released from previously infected cells, cGAMP in bystander cells can activate STING, IRF4-dependent antiviral pathways and IFN production independent of signaling mediated by viral nucleic acids. These propagating innate immune anti-viral responses appear to limit the spread of infection.

Viral nucleic acids signal through intracellular PRRs including TLR3, 7, 8 and 9 in the endosomes and AIM-2, RIG-1, STING and cGAS in the cytosol [68]. During infection with viruses or bacteria, however, activation of NADPH oxidases promotes oxidative damage to host cell DNA, releasing host ssDNA or dsDNA into the cytoplasm. Recognizing the ssDNA or dsDNA, the DNA sensor STING primes PRRs to orchestrate an amplified innate immune response, which is apparent when ATM-signaling for DNA repair is mutated, absent or silenced [69]. Although demonstrated in mononuclear cells, there is evidence for parallel mechanisms in mucosal epithelial cells [70]. Damaged host DNA appears to function as a danger signal resulting in rapid and robust responses to microbial and environmental challenges.

In squamous mucosal epithelium including cells from the vaginal wall, innate immune sensors of viral infection of squamous epithelial cells include Toll-like receptors TLR2 and TLR3, RIG-I, and MDA-5. These sensors can be down-regulated by the virion host shutoff protein of herpes simplex virus type 2 [71]. Reduction in sensing dsDNA attenuates the IFN- β antiviral response. RIG-1 and MDA-5 contain a helicase and two CARD-domains. Found in the cytoplasm, the RIG-like receptors bind viral nucleic acids and complex with mitochondria. At the mitochondria, complex signaling pathways result in activation of antiviral transcriptional responses characteristic of NF- κ B and type I interferon.

5. The inflammasome

Select members of the NOD receptor (NLRs) family (of the 22 members) recognize intracellular pathogens, both bacterial and viral [72]. As intracellular PRRs, NOD1/2 are expressed in oral epithelium and appear to act synergistically with TLRs to promote antibacterial responses [32]. Activation of the NLRs regulates inflammatory and apoptotic responses. NLR activity is regulated by complex interactions with as many as 15 other proteins known to regulate the innate immune response, including RIG-1, a negative regulator, and CARD9, a positive regulator. Atg16L1 contributes to induction and regulation of autophagy. The NRPs also include NALP1 molecules, which oligomerize; the oligomer activates the caspase-1 cascade to produce pro-inflammatory cytokines including IL-1 β and

IL-18 [73]. Since the transcriptional product is proinflammatory cytokines, the NALP1 oligomer was termed the “inflammasome.” Two other NLR subsets, NLRP3 and NLRC4 are also inflammasomes.

Activation of one of four PRR family members (AIM2, NLRC4 or IPAF, NLRP1, and NLRP3) initiates inflammasome formation (Figure 2). NLRC4 inflammasome is involved in disease tolerance mediated by commensal microbiome *E. coli* [74]. Inflammasome activators cause simultaneous release of proinflammatory IL-1 α and IL-1 β [75].

Inflammasomes are organized into sensor proteins such as the NLRs and an adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD-like domain) [76]. The sensor proteins discriminate between PAMPs such as peptidoglycan fragments (NLRP1), microbial proteins (NLRC4) and dsDNA from host or microbial sources (AIM2). For some microbes such as *P. gingivalis*, there appears to be a survival advantage to activate the inflammasome and dampen the response in the oral epithelium. PAMP engagement is the first required signal to activate the inflammasome and produce proinflammatory cytokines [77]. A second signal such as extracellular ATP (eATP) is needed for inflammasome assembly, caspase-1 processing of the pro-cytokine, and secretion of the mature active cytokine. eATP is released from dead and dying cells and tissues perhaps as a consequence of infection by invasive pathogens such as *Chlamydia trachomatis* [78] and is therefore considered a danger signal. The eATP binds to P2X7, a purinergic receptor expressed on most squamous epithelial cells. When oral epithelial cells are incubated with *P. gingivalis* or lipopolysaccharide and eATP, NALP3 is activated, binds caspase-1, and promotes secretion of active IL-1 β [77]. *P. gingivalis* is necessary to upregulate pro-IL-1 β to cause secretion of the active cytokine. In oral epithelial cells, engagement of eATP by P2X7 receptors appears to signal for activation of NADPH oxidase and sustained production of antimicrobial reactive oxygen species (ROS) within the cells [79]. By secreting nucleoside-diphosphate-kinase (Ndk), which can reversibly dephosphorylate ATP, *P. gingivalis* has evolved a persistence mechanism, dampening the production of antimicrobial ROS. Driven by eATP, oral epithelial cells release high-mobility group protein B1 (HMGB1) from the chromatin of healthy cells, a proinflammatory danger signal [80]). *P. gingivalis* reduces release of HMGB1 in an Ndk- and caspase-1 dependent manner, perhaps reflecting the suppression of innate autonomous epithelial immune mechanisms.

The inflammasome requires recognition of PAMPs and a second signal to activate downstream signaling. For example, the periodontal bacterium *Fusobacterium nucleatum* infects oral epithelial cells in vitro and triggers activation of the inflammasome without eATP or other second signals [81]. When eATP serves as a second signal, caspase-1 activates and the cells release mature IL-1 β , HMGB1 and ASC. Whether activation of the inflammasome reflects epithelial cell autonomous immunity or a mechanism to propagate danger signals to proximal cells appears to be an unanswered question. Certainly activation of ROS is likely to thwart the growth of intracellular pathogens, but the relative contributions of each of the concurrently acting innate intracellular antimicrobial activities remains to be established.

When unchecked, activation of the inflammasome can cause cell death, which may prove to be therapeutic with some infections. In response to PAMPs, exuberant inflammasome

activation leading to activation of caspase-1 and 4 can cause Caspase-1/Caspase-4- and NLRP3-dependent inflammatory cell death in human urothelial cells [82]. Mediated by uropathogenic *E. coli* (UPEC) α -hemolysin (HlyA), cell death can cause exfoliation of the bladder lining and reduced bacterial burdens in vivo. This process likely purges the urinary epithelium of attached and internalized UPEC and facilitates the regeneration of a healthier epithelial barrier. Similarly, the gut mucosal epithelium is susceptible to invasion by *Salmonella enterica* serovar Typhimurium [83]. Under control of inflammasome components, infected enterocytes are expelled into the lumen, restricting the intracellular proliferation of the pathogen. In response to *S. Typhimurium*, intestinal epithelial cells undergo pyroptotic cell death activation of the Caspase-4 inflammasome, which reduces pathogen burdens in the intestinal mucosa [84]. The dispersion of expelled enterocytes may be aided by fluid flow from inflammasome-dependent goblet cell secretion of mucins [85]. The process of expulsion of infected cells appears to be independent of concomitant secretion of immunomodulatory IL-1 α/β and IL-1 β and a generalizable innate immune mechanism to purge pathogens from mucosal epithelia. Whether pyroptosis occurs in squamous mucosal epithelia has not been reported.

6. Effector arm of innate intraepithelial immunity

The effector arm of epithelial autonomous innate immunity centers on the expression of antimicrobial proteins and peptides (AMPs) in addition to ROS production. Some AMPs appear to be “assigned” to protect the epithelial cell against microbes in the endosomes, where most invasive bacteria first appear. After bacteria or fungi escape from the endosomes, AMPs in the cytoplasm also seem to protect the cell. AMPs released or secreted into the pericellular environment may limit the growth of microbes and fungi in the biofilms that approximate the epithelial cell. Purified hBD-3 and LL-37, for example, are broad spectrum AMPs that show antibiofilm activity in vitro [86]. It is unclear, however, whether their release from mucosal epithelial cells serves an innate autonomous immune function to protect mucosal epithelial cells from pathogens in vitro or in vivo. A notable exception may be rabbit vaginal fluid hemoglobin alpha peptide (RVFHb α P), which is produced by vaginal epithelial cells and appears to function in vaginal fluid to protect endocervical cells against invasive pathogens [87].

As described, a key epithelial response to infection by many mucosal pathogens is the production and secretion of IL-1 α/β and IL-1 β [23, 84] and IL-18 [2, 3]. After release from mucosal epithelial cells, these cytokines are bound by specific receptors (i.e., IL-1R) on the same or neighboring cells. When the same or neighboring epithelial cells release and bind select cytokines, an autocrine loop establishes that signals for increased expression of AMPs (Figure 1). The increased production of S100A8/A9, for example, which is inhibited by pretreatment of the cells with IL-1R antagonist, augments resistance of the cells against invasive pathogens such as *Listeria* and *Salmonella* [23]. Hence, cell autonomous immunity can be stimulated by encounters with pathogens but without reliance on cells of non-epithelial lineages.

Constitutively expressed in the cytoplasm of healthy gingival epithelial cells [88], calprotectin (S100A8/A9) appears to protect the cytoplasm against invading pathogens

including *Salmonella*, *Listeria* and *P. gingivalis* [89–91]. During periodontitis in human patients, S100A8/A9 is upregulated in the inflamed epithelium of the gingival crevice [92]. As we reported, pathogens and autocrine IL-1 α produced by [93–95] activate and stimulate DNA binding by the transcription factor C/EBP β which upregulates S100A8/A9 (Figure 1). S100A8/A9 upregulation may also reflect an NF- κ B-dependent response of epithelial cells to selected periodontal pathogens in vitro [96]. When upregulated, S100A8/A9 increases antimicrobial activity in the cytoplasm of mucosal epithelial cells against invasive bacteria, augmenting cell autonomous immunity [23].

S100A8/A9, like many AMPs, appears to function extracellularly in vitro. Purified S100A8/A9 shows antimicrobial activity against a wide spectrum of bacteria and fungi. Upon release from epithelial cells and neutrophils, S100A8/A9 is a major AMP in neutrophil extracellular traps (NETs), which appear to contain and neutralize the spread of bacterial pathogens [97, 98]. In periodontitis lesions, NETs are characterized as a mesh of citrullinated histone H3 [99, 100] and extruded DNA [101, 102] in complex with S100A8/A9 [103]. In mouse models, S100A8/A9 release into the extracellular environment of the gut suppresses commensal bacteria, facilitating the outgrowth of “true” pathogens, such as *Salmonella* [104–106]. Associated inflammation in the gut upregulates epithelial S100A8/A9 [107]. Failure to upregulate S100A8/A9 affects the oral salivary microbiome, changing the course of periapical lesions at the roots of teeth [108]. Whereas “alarmins” act to recruit and activate inflammatory cells, S100A8/A9-dependent intracellular and extracellular AMP activities would appear to protect against and mitigate periodontal infection. The balance of S100A8/A9 proinflammatory and AMP activities during periodontitis, however, remains to be determined.

S100A8/A9 polymorphisms, like those in IL-1 β [109], are among the few human genetic variants shown to be associated with periodontitis. Consistent with a protective role for the S100A8/A9 complex in periodontal diseases, single nucleotide polymorphisms (i.e., SNP rs3795391, A > G) in the S100A8 upstream promoter are associated with increased overall risk of periodontitis [110] and susceptibility to aggressive periodontitis within a family [111] and across a population [112]. The rs3795391, A > G polymorphism is less prevalent in patients with aggressive periodontitis than the AA genotype, although plasma concentrations of S100A8/A9 were similar to patients with SNP rs 3806232 [112]. The copy number of transcripts for the two S100A8 promoter SNPs has not been reported, although differences may be expected. Whereas the AA genotype may increase susceptibility, the S100A8/A9 GA and GG genotypes appear protective against severe disease and likely contribute to innate protection during initiation of periodontitis and bone loss.

LL-37 is a potent antimicrobial peptide on a molar basis, which is expressed widely among mucosal epithelial cells [113]. This peptide product of the pro-protein cathelicidin antimicrobial protein (CAMP; hCAP18) shows activity against many bacteria, fungi and viruses (examples follow). Reported susceptible viruses include respiratory syncytial virus [114], influenza A [115], and HIV-1 [116]. Many species are both susceptible and have also evolved defensive mechanisms. For example, the mucosal commensal yeast and opportunistic pathogen, *Candida albicans*, can be inactivated by LL-37 but the yeast uses secreted aspartate proteases to degrade this AMP [117]. Similarly, *Chlamydia trachomatis*, a

Gram-negative pathogen, infects the lower genital tract and is antagonized in part by LL-37 produced by the urogenital epithelial cells [118]. Chlamydia in turn secretes a serine protease that inactivates LL-37. By degrading intracellular AMPs using the serine protease, invaded Chlamydia persists within epithelial cells. Epithelial-derived cathelicidin can be ineffective against *E. coli* causing urinary tract infections; strains that are more resistant to the AMP cause more severe infections [119]. LL-37 can also exacerbate infection. For example, *E. coli* cystitis is less severe in cathelicidin-deficient than wild-type mice [120]. These seemingly contradictory results need to be reconciled. Whereas LL-37 is an effective AMP against *Neisseria gonorrhoeae* in vitro, LL-37 is downregulated at the transcriptional and translational levels during live bacterial infection of cervical epithelial cells. Such mechanisms may facilitate a survival benefit to *N. gonorrhoeae*.

Upon complexing with TLR3, LL-37 can traffic to early endosomes in lung epithelial cells for example [121]. In morbus Kostmann, a severe congenital neutropenia, cathelicidin/LL-37 is not detectable in saliva or plasma and the patients show severe periodontal disease [122]. Normalizing neutrophil counts are not sufficient to maintain or restore periodontal health [123] suggesting that deficiency of LL-37 places the periodontal tissues at risk of infection. Whether oral mucosal epithelial cells fail to produce LL-37 in morbus Kostmann disease has not been reported and so it is unclear that LL-37 in the gingival crevicular fluid, which bathes the gingival sulcular epithelium, contributes to epithelial autonomous immunity.

In contrast, hBD-1 is constitutively expressed by genital tract epithelium. An evaluation of five known DEFB1 gene polymorphisms suggested that hBD1 mitigates risk of HPV infection in women [124]). Patients with periodontitis were found to express a polymorphism in a single SNP of DEFB1 (−1654(V38I) [125]. hBD-3 is expressed by the epithelium that lines the mammalian respiratory tree and other mucosal epithelia. Copy number variations in DEFB103 affects the composition of key pathogens in the nasopharyngeal microbiome but does not affect the risk of respiratory infections such as recurrent otitis media [126]. Copy number variations in DEFB4, the gene encoding hBD-2, and serum hBD-2 were studied for their contribution to risk of periodontitis [127]. Decreased DEFB4 copy number was accompanied by reduced serum hBD-2 and increased severity of periodontitis. Seemingly conflicting, DEFB4 copy number was higher in patients with Crohn's disease, who are at risk of severe periodontitis, than in unaffected individuals [128]. Unlike LL-37 and S100A8/A9, hBDs have not been reported to show antimicrobial function within mucosal epithelial cells, except for a possible restriction against HIV-1 replication [129]. Whether these AMPs contribute to the ability of mucosal epithelial cells to control surface biofilms is not clear, but disease manifestations are apparently affected.

7. Conclusions

Mucosal epithelial cells express an autonomous innate immune response that controls the overgrowth of invaded bacteria, mitigating the harmful effects of the bacteria carried within. This protective response is epithelial cell autonomous and does not rely on other arms of the immune response occurring in the external environment. It is innate, and does not rely on prior experience with the infectious agents. The response is surprisingly sophisticated, and includes extra- and intracellular recognition of specific molecular features of adhering and

invading bacteria, signaling mechanisms that transcriptionally regulate expression of the effector proteins. Epithelial cell autonomous innate immunity also “respects” the social biology and strategies of the invading bacteria to achieve symbiosis. That the epithelial cell can tailor a response to the species of invader suggests a long and successful evolutionary path. Indeed, for bacteria themselves and lower primitive invertebrates, the cell autonomous immune response is the primary protective mechanism against pathogens.

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References

1. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol*. 2010; 10:159–169. [PubMed: 20182457]
2. Levy M, Thaïss CA, Zeevi D, Dohnalova L, Zilberman-Schapira G, Mahdi JA, et al. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell*. 2015; 163:1428–1443. [PubMed: 26638072]
3. Nowarski R, Jackson R, Gagliani N, de Zoete MR, Palm NW, Bailis W, et al. Epithelial IL-18 equilibrium controls barrier function in colitis. *Cell*. 2015; 163:1444–1456. [PubMed: 26638073]
4. Beutler B, Jiang Z, Georgel P, Crozat K, Croker B, Rutschmann S, et al. Genetic analysis of host resistance: Toll-like receptor signaling and immunity at large. *Annu Rev Immunol*. 2006; 24:353–389. [PubMed: 16551253]
5. Randow F, MacMicking JD, James LC. Cellular self-defense: how cell-autonomous immunity protects against pathogens. *Science*. 2013; 340:701–706. [PubMed: 23661752]
6. MacMicking JD. Cell-autonomous effector mechanisms against *Mycobacterium tuberculosis*. *Cold Spring Harb Perspect Med*. 2014;4.
7. Staskawicz BJ. Genetics of plant-pathogen interactions specifying plant disease resistance. *Plant Physiol*. 2001; 125:73–76. [PubMed: 11154300]
8. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. *Science*. 2010; 327:167–170. [PubMed: 20056882]
9. Kreth J, Zhang Y, Herzberg MC. Streptococcal antagonism in oral biofilms: *Streptococcus sanguinis* and *Streptococcus gordonii* interference with *Streptococcus mutans*. *J Bacteriol*. 2008; 190:4632–4640. [PubMed: 18441055]
10. Kreth J, Vu H, Zhang Y, Herzberg MC. Characterization of hydrogen peroxide-induced DNA release by *Streptococcus sanguinis* and *Streptococcus gordonii*. *J Bacteriol*. 2009; 191:6281–6291. [PubMed: 19684131]
11. Ross KF, Ronson CW, Tagg JR. Isolation and characterization of the lantibiotic salivaricin A and its structural gene salA from *Streptococcus salivarius* 20P3. *Appl Environ Microbiol*. 1993; 59:2014–2021. [PubMed: 8357242]
12. Groeger SE, Meyle J. Epithelial barrier and oral bacterial infection. *Periodontol* 2000. 2015; 69:46–67. [PubMed: 26252401]
13. Zhang K, Horneff MW, Dupont A. The intestinal epithelium as guardian of gut barrier integrity. *Cell Microbiol*. 2015; 17:1561–1569. [PubMed: 26294173]
14. Sonnenberg GF, Artis D. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. *Nat Med*. 2015; 21:698–708. [PubMed: 26121198]
15. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. *Nat Rev Immunol*. 2011; 11:445–456. [PubMed: 21681197]
16. Martin FJ, Prince AS. TLR2 regulates gap junction intercellular communication in airway cells. *J Immunol*. 2008; 180:4986–4993. [PubMed: 18354224]

17. Kasper CA, Sorg I, Schmutz C, Tschon T, Wischnewski H, Kim ML, et al. Cell-cell propagation of NF-kappaB transcription factor and MAP kinase activation amplifies innate immunity against bacterial infection. *Immunity*. 2010; 33:804–816. [PubMed: 21093316]
18. Tran Van Nhieu G, Clair C, Bruzzone R, Mesnil M, Sansonetti P, Combettes L. Connexin-dependent inter-cellular communication increases invasion and dissemination of *Shigella* in epithelial cells. *Nat Cell Biol*. 2003; 5:720–706. [PubMed: 12844145]
19. Velasquez Almonacid LA, Tafuri S, Dipineto L, Matteoli G, Fiorillo E, Della Morte R, et al. Role of connexin-43 hemichannels in the pathogenesis of *Yersinia enterocolitica*. *Vet J*. 2009; 182:452–457. [PubMed: 18824377]
20. Hornef MW, Fulde M. Ontogeny of intestinal epithelial innate immune responses. *Front Immunol*. 2014; 5:474. [PubMed: 25346729]
21. Reikine S, Nguyen JB, Modis Y. Pattern recognition and signaling mechanisms of RIG-I and MDA5. *Front Immunol*. 2014; 5:342. [PubMed: 25101084]
22. Brubaker SW, Bonham KS, Zanoni I, Kagan JC. Innate immune pattern recognition: a cell biological perspective. *Annu Rev Immunol*. 2015; 33:257–290. [PubMed: 25581309]
23. Sorenson BS, Khammanivong A, Guenther BD, Ross KF, Herzberg MC. IL-1 receptor regulates S100A8/A9-dependent keratinocyte resistance to bacterial invasion. *Mucosal Immunol*. 2012; 5:66–75. [PubMed: 22031183]
24. Takeuchi H, Furuta N, Morisaki I, Amano A. Exit of intracellular *Porphyromonas gingivalis* from gingival epithelial cells is mediated by endocytic recycling pathway. *Cell Microbiol*. 2011; 13:677–691. [PubMed: 21155963]
25. Benjamin JL, Sumpter R, Levine B, Hooper LV Jr. Intestinal epithelial autophagy is essential for host defense against invasive bacteria. *Cell Host Microbe*. 2013; 13:723–734. [PubMed: 23768496]
26. Barbalat R, Lau L, Locksley RM, Barton GM. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat Immunol*. 2009; 10:1200–1207. [PubMed: 19801985]
27. Dietrich N, Lienenklaus S, Weiss S, Gekara NO. Murine toll-like receptor 2 activation induces type I interferon responses from endolysosomal compartments. *PLoS One*. 2010; 5:e10250. [PubMed: 20422028]
28. Triantafilou K, Vakakis E, Richer EA, Evans GL, Villiers JP, Triantafilou M. Human rhinovirus recognition in non-immune cells is mediated by Toll-like receptors and MDA-5, which trigger a synergetic pro-inflammatory immune response. *Virulence*. 2011; 2:22–29. [PubMed: 21224721]
29. Marsden V, Donaghy H, Bertram KM, Harman AN, Nasr N, Keoshkerian E, et al. Herpes simplex virus type 2-infected dendritic cells produce TNF-alpha, which enhances CCR5 expression and stimulates HIV production from adjacent infected cells. *J Immunol*. 2015; 194:4438–4445. [PubMed: 25840914]
30. Promsudthi A, Poomsawat S, Limsricharoen W. The role of Toll-like receptor 2 and 4 in gingival tissues of chronic periodontitis subjects with type 2 diabetes. *J Periodontal Res*. 2014; 49:346–354. [PubMed: 23844856]
31. Lu Q, Darveau RP, Samaranayake LP, Wang CY, Jin L. Differential modulation of human {beta}-defensins expression in human gingival epithelia by *Porphyromonas gingivalis* lipopolysaccharide with tetra- and penta-acylated lipid A structures. *Innate Immun*. 2009; 15:325–335. [PubMed: 19675119]
32. Uehara A, Takada H. Synergism between TLRs and NOD1/2 in oral epithelial cells. *J Dent Res*. 2008; 87:682–686. [PubMed: 18573991]
33. Sugawara Y, Uehara A, Fujimoto Y, Kusumoto S, Fukase K, Shibata K, et al. Toll-like receptors, NOD1, and NOD2 in oral epithelial cells. *J Dent Res*. 2006; 85:524–529. [PubMed: 16723649]
34. Lange MJ, Lasiter JC, Misfeldt ML. Toll-like receptors in tonsillar epithelial cells. *Int J Pediatr Otorhinolaryngol*. 2009; 73:613–621. [PubMed: 19167100]
35. Semlali A, Witoled C, Alanazi M, Rouabhia M. Whole cigarette smoke increased the expression of TLRs, HBDs, and proinflammatory cytokines by human gingival epithelial cells through different signaling pathways. *PLoS One*. 2012; 7:e52614. [PubMed: 23300722]

36. Mori Y, Yoshimura A, Ukai T, Lien E, Espevik T, Hara Y. Immunohistochemical localization of Toll-like receptors 2 and 4 in gingival tissue from patients with periodontitis. *Oral Microbiol Immunol.* 2003; 18:54–58. [PubMed: 12588460]
37. Ren L, Leung WK, Darveau RP, Jin L. The expression profile of lipopolysaccharide-binding protein, membrane-bound CD14, and toll-like receptors 2 and 4 in chronic periodontitis. *J Periodontol.* 2005; 76:1950–1959. [PubMed: 16274315]
38. Janardhanam SB, Prakasam S, Swaminathan VT, Kodumudi KN, Zunt SL, Srinivasan M. Differential expression of TLR-2 and TLR-4 in the epithelial cells in oral lichen planus. *Arch Oral Biol.* 2012; 57:495–502. [PubMed: 22119043]
39. Soto C, Bugueno I, Hoare A, Gonzalez S, Venegas D, Salinas D, et al. The *Porphyromonas gingivalis* O antigen is required for inhibition of apoptosis in gingival epithelial cells following bacterial infection. *J Periodontal Res.* 2015
40. Wang Q, Sztukowska M, Ojo A, Scott DA, Wang H, Lamont RJ. FOXO responses to *Porphyromonas gingivalis* in epithelial cells. *Cell Microbiol.* 2015; 17:1605–1617. [PubMed: 25958948]
41. Fichorova RN, Cronin AO, Lien E, Anderson DJ, Ingalls RR. Response to *Neisseria gonorrhoeae* by cervicovaginal epithelial cells occurs in the absence of toll-like receptor 4-mediated signaling. *J Immunol.* 2002; 168:2424–2432. [PubMed: 11859134]
42. Herbst-Kralovetz MM, Quayle AJ, Ficarra M, Greene S, Rose WA 2nd, Chesson R, et al. Quantification and comparison of toll-like receptor expression and responsiveness in primary and immortalized human female lower genital tract epithelia. *Am J Reprod Immunol.* 2008; 59:212–224. [PubMed: 18201283]
43. Rose WA 2nd, McGowin CL, Spagnuolo RA, Eaves-Pyles TD, Popov VL, Pyles RB. Commensal bacteria modulate innate immune responses of vaginal epithelial cell multilayer cultures. *PLoS One.* 2012; 7:e32728. [PubMed: 22412914]
44. Dusio GF, Cardani D, Zanobbio L, Mantovani M, Luchini P, Battini L, et al. Stimulation of TLRs by LMW-HA induces self-defense mechanisms in vaginal epithelium. *Immunol Cell Biol.* 2011; 89:630–639. [PubMed: 21102537]
45. Frew L, Makieva S, McKinlay AT, McHugh BJ, Doust A, Norman JE, et al. Human cathelicidin production by the cervix. *PLoS One.* 2014; 9:e103434. [PubMed: 25089904]
46. Kumar RB, Maher DM, Herzberg MC, Southern PJ. Expression of HIV receptors, alternate receptors and co-receptors on tonsillar epithelium: implications for HIV binding and primary oral infection. *Virology.* 2006; 3:25. [PubMed: 16600047]
47. Li S, Dong G, Moschidis A, Ortiz J, Benakanakere MR, Kinane DF, et al. *P. gingivalis* modulates keratinocytes through FOXO transcription factors. *PLoS One.* 2013; 8:e78541. [PubMed: 24265696]
48. Giacaman RA, Asrani AC, Gebhard KH, Dietrich EA, Vacharaksa A, Ross KF, et al. *Porphyromonas gingivalis* induces CCR5-dependent transfer of infectious HIV-1 from oral keratinocytes to permissive cells. *Retrovirology.* 2008; 5:29. [PubMed: 18371227]
49. Giacaman RA, Nobbs AH, Ross KF, Herzberg MC. *Porphyromonas gingivalis* selectively up-regulates the HIV-1 coreceptor CCR5 in oral keratinocytes. *J Immunol.* 2007; 179:2542–2550. [PubMed: 17675516]
50. Giacaman RA, Asrani AC, Ross KF, Herzberg MC. Cleavage of protease-activated receptors on an immortalized oral epithelial cell line by *Porphyromonas gingivalis* gingipains. *Microbiology.* 2009; 155:3238–3246. [PubMed: 19608609]
51. Vacharaksa A, Asrani AC, Gebhard KH, Fasching CE, Giacaman RA, Janoff EN, et al. Oral keratinocytes support non-replicative infection and transfer of harbored HIV-1 to permissive cells. *Retrovirology.* 2008; 5:66. [PubMed: 18637194]
52. Dietrich EA, Gebhard KH, Fasching CE, Giacaman RA, Kappes JC, Ross KF, et al. Short communication: HIV type 1 escapes inactivation by saliva via rapid escape into oral epithelial cells. *AIDS Res Hum Retroviruses.* 2012; 28:1574–1578. [PubMed: 22077822]
53. Hache G, Mansky LM, Harris RS. Human APOBEC3 proteins, retrovirus restriction, and HIV drug resistance. *AIDS Rev.* 2006; 8:148–157. [PubMed: 17078485]
54. Harris RS, Dudley JP. APOBECs and virus restriction. *Virology.* 2015; 479–480:131–145.

55. Mbisa JL, Bu W, Pathak VK. APOBEC3F and APOBEC3G inhibit HIV-1 DNA integration by different mechanisms. *J Virol.* 2010; 84:5250–5259. [PubMed: 20219927]
56. Sadler HA, Stenglein MD, Harris RS, Mansky LM. APOBEC3G contributes to HIV-1 variation through sublethal mutagenesis. *J Virol.* 2010; 84:7396–7404. [PubMed: 20463080]
57. Pace C, Keller J, Nolan D, James I, Gaudieri S, Moore C, et al. Population level analysis of human immunodeficiency virus type 1 hypermutation and its relationship with APOBEC3G and vif genetic variation. *J Virol.* 2006; 80:9259–9269. [PubMed: 16940537]
58. Dapp MJ, Holtz CM, Mansky LM. Concomitant lethal mutagenesis of human immunodeficiency virus type 1. *J Mol Biol.* 2012; 419:158–170. [PubMed: 22426127]
59. Dinh MH, Anderson MR, McRaven MD, Cianci GC, McCoombe SG, Kelley ZL, et al. Visualization of HIV-1 interactions with penile and foreskin epithelia: clues for female-to-male HIV transmission. *PLoS Pathog.* 2015; 11:e1004729. [PubMed: 25748093]
60. Zou J, Li W, Misra A, Yue F, Song K, Chen Q, et al. The viral restriction factor tetherin prevents leucine-rich pentatricopeptide repeat-containing protein (LRPPRC) from association with beclin 1 and B-cell CLL/lymphoma 2 (Bcl-2) and enhances autophagy and mitophagy. *J Biol Chem.* 2015; 290:7269–7279. [PubMed: 25631043]
61. Tabah AA, Tardif K, Mansky LM. Anti-HIV-1 activity of Trim 37. *J Gen Virol.* 2014; 95:960–967. [PubMed: 24317724]
62. Tugizov SM, Herrera R, Veluppillai P, Greenspan D, Soros V, Greene WC, et al. Differential transmission of HIV traversing fetal oral/intestinal epithelia and adult oral epithelia. *J Virol.* 2012; 86:2556–2570. [PubMed: 22205732]
63. Herrera R, Morris M, Rosbe K, Feng Z, Weinberg A, Tugizov S. Human beta-defensins 2 and –3 coinfect with human immunodeficiency virus via heparan sulfate proteoglycans and reduce infectivity of intracellular virions in tonsil epithelial cells. *Virology.* 2016; 487:172–187. [PubMed: 26539799]
64. Gentili M, Kowal J, Tkach M, Satoh T, Lahaye X, Conrad C, et al. Transmission of innate immune signaling by packaging of cGAMP in viral particles. *Science.* 2015; 349:1232–1236. [PubMed: 26229115]
65. Bridgeman A, Maelfait J, Davenne T, Partridge T, Peng Y, Mayer A, et al. Viruses transfer the antiviral second messenger cGAMP between cells. *Science.* 2015; 349:1228–1232. [PubMed: 26229117]
66. Ablasser A, Goldeck M, Cavlar T, Deimling T, Witte G, Rohl I, et al. cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. *Nature.* 2013; 498:380–384. [PubMed: 23722158]
67. Ablasser A, Schmid-Burgk JL, Hemmerling I, Horvath GL, Schmidt T, Latz E, et al. Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. *Nature.* 2013; 503:530–534. [PubMed: 24077100]
68. Crump KE, Sahingur SE. Microbial nucleic acid sensing in oral and systemic diseases. *J Dent Res.* 2016; 95:17–25. [PubMed: 26438211]
69. Hartlova A, Erttmann SF, Raffi FA, Schmalz AM, Resch U, Anugula S, et al. DNA damage primes the type I interferon system via the cytosolic DNA sensor STING to promote anti-microbial innate immunity. *Immunity.* 2015; 42:332–343. [PubMed: 25692705]
70. Fang L, Choudhary S, Tian B, Boldogh I, Yang C, Ivanciuc T, et al. Ataxia telangiectasia mutated kinase mediates NF-kappaB serine 276 phosphorylation and interferon expression via the IRF7-RIG-I amplification loop in paramyxovirus infection. *J Virol.* 2015; 89:2628–2642. [PubMed: 25520509]
71. Yao XD, Rosenthal KL. Herpes simplex virus type 2 virion host shutoff protein suppresses innate dsRNA antiviral pathways in human vaginal epithelial cells. *J Gen Virol.* 2011; 92:1981–1993. [PubMed: 21632561]
72. Moreira LO, Zamboni DS. NOD1 and NOD2 signaling in infection and inflammation. *Front Immunol.* 2012; 3:328. [PubMed: 23162548]
73. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell.* 2002; 10:417–426. [PubMed: 12191486]

74. Schieber AM, Lee YM, Chang MW, Leblanc M, Collins B, Downes M, et al. Disease tolerance mediated by microbiome *E. coli* involves inflammasome and IGF-1 signaling. *Science*. 2015; 350:558–563. [PubMed: 26516283]
75. Gross O, Yazdi AS, Thomas CJ, Masin M, Heinz LX, Guarda G, et al. Inflammasome activators induce interleukin-1 α secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity*. 2012; 36:388–400. [PubMed: 22444631]
76. Santana PT, Martel J, Lai HC, Perfettini JL, Kanellopoulos JM, Young JD, et al. Is the inflammasome relevant for epithelial cell function? *Microbes Infect*. 2015
77. Yilmaz O, Sater AA, Yao L, Koutouzis T, Pettengill M, Ojcius DM. ATP-dependent activation of an inflammasome in primary gingival epithelial cells infected by *Porphyromonas gingivalis*. *Cell Microbiol*. 2010; 12:188–198. [PubMed: 19811501]
78. Pettengill MA, Marques-da-Silva C, Avila ML, d'Arc dos Santos Oliveira S, Lam VW, Ollawa I, et al. Reversible inhibition of *Chlamydia trachomatis* infection in epithelial cells due to stimulation of P2X(4) receptors. *Infect Immun*. 2012; 80:4232–4238. [PubMed: 22988022]
79. Choi CH, Spooner R, DeGuzman J, Koutouzis T, Ojcius DM, Yilmaz O. *Porphyromonas gingivalis*-nucleoside-diphosphate-kinase inhibits ATP-induced reactive-oxygen-species via P2X7 receptor/NADPH-oxidase signalling and contributes to persistence. *Cell Microbiol*. 2013; 15:961–976. [PubMed: 23241000]
80. Johnson L, Atanasova KR, Bui PQ, Lee J, Hung SC, Yilmaz O, et al. *Porphyromonas gingivalis* attenuates ATP-mediated inflammasome activation and HMGB1 release through expression of a nucleoside-diphosphate kinase. *Microbes Infect*. 2015; 17:369–377. [PubMed: 25828169]
81. Bui FQ, Johnson L, Roberts J, Hung SC, Lee J, Atanasova KR, et al. *Fusobacterium nucleatum* infection of gingival epithelial cells leads to NLRP3 inflammasome-dependent secretion of IL-1 β and the danger signals ASC and HMGB1. *Cell Microbiol*. 2015
82. Nagamatsu K, Hannan TJ, Guest RL, Kostakioti M, Hadjifrangiskou M, Binkley J, et al. Dysregulation of *Escherichia coli* α -hemolysin expression alters the course of acute and persistent urinary tract infection. *Proc Natl Acad Sci U S A*. 2015; 112:E871–E880. [PubMed: 25675528]
83. Muller AJ, Kaiser P, Dittmar KE, Weber TC, Haueter S, Endt K, et al. *Salmonella* gut invasion involves TTSS-2-dependent epithelial traversal, basolateral exit, and uptake by epithelium-sampling lamina propria phagocytes. *Cell Host Microbe*. 2012; 11:19–32. [PubMed: 22264510]
84. Knodler LA, Crowley SM, Sham HP, Yang H, Wrande M, Ma C, et al. Noncanonical inflammasome activation of caspase-4/caspase-11 mediates epithelial defenses against enteric bacterial pathogens. *Cell Host Microbe*. 2014; 16:249–256. [PubMed: 25121752]
85. Wlodarska M, Thaiss CA, Nowarski R, Henao-Mejia J, Zhang JP, Brown EM, et al. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell*. 2014; 156:1045–1059. [PubMed: 24581500]
86. Batoni G, Maisetta G, Esin S. Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. *Biochim Biophys Acta*. 2015
87. Patgaonkar M, Aranha C, Bhonde G, Reddy KV. Identification and characterization of antimicrobial peptides from rabbit vaginal fluid. *Vet Immunol Immunopathol*. 2011; 139:176–186. [PubMed: 21047689]
88. Ross KF, Herzberg MC. Calprotectin expression by gingival epithelial cells. *Infect Immun*. 2001; 69:3248–3254. [PubMed: 11292747]
89. Nisapakultorn K, Ross KF, Herzberg MC. Calprotectin expression in vitro by oral epithelial cells confers resistance to infection by *Porphyromonas gingivalis*. *Infect Immun*. 2001; 69:4242–4247. [PubMed: 11401960]
90. Zaia AA, Sappington KJ, Nisapakultorn K, Chazin WJ, Dietrich EA, Ross KF, et al. Subversion of antimicrobial calprotectin (S100A8/S100A9 complex) in the cytoplasm of TR146 epithelial cells after invasion by *Listeria monocytogenes*. *Mucosal Immunol*. 2009; 2:43–53. [PubMed: 19079333]
91. Champaboon C, Sappington KJ, Guenther BD, Ross KF, Herzberg MC. Calprotectin S100A9 calcium-binding loops I and II are essential for keratinocyte resistance to bacterial invasion. *J Biol Chem*. 2009; 284:7078–7090. [PubMed: 19122197]

92. Eversole LR, Miyasaki KT, Christensen RE. Keratinocyte expression of calprotectin in oral inflammatory mucosal diseases. *J Oral Pathol Med.* 1993; 22:303–307. [PubMed: 8229866]
93. Bando M, Hiroshima Y, Kataoka M, Herzberg MC, Ross KF, Shinohara Y, et al. Modulation of calprotectin in human keratinocytes by keratinocyte growth factor and interleukin-1alpha. *Immunol Cell Biol.* 2010; 88:328–333. [PubMed: 20065999]
94. Bando M, Zou X, Hiroshima Y, Kataoka M, Ross KF, Shinohara Y, et al. Mechanism of interleukin-1alpha transcriptional regulation of S100A9 in a human epidermal keratinocyte cell line. *Biochim Biophys Acta.* 2013; 1829:954–962. [PubMed: 23563247]
95. Hiroshima Y, Bando M, Kataoka M, Inagaki Y, Herzberg MC, Ross KF, et al. Regulation of antimicrobial peptide expression in human gingival keratinocytes by interleukin-1alpha. *Arch Oral Biol.* 2011; 56:761–767. [PubMed: 21316034]
96. Milward MR, Chapple IL, Wright HJ, Millard JL, Matthews JB, Cooper PR. Differential activation of NF-kappaB and gene expression in oral epithelial cells by periodontal pathogens. *Clin Exp Immunol.* 2007; 148:307–324. [PubMed: 17355248]
97. Achouiti A, Vogl T, Urban CF, Rohm M, Hommes TJ, van Zoelen MA, et al. Myeloid-related protein-14 contributes to protective immunity in gram-negative pneumonia derived sepsis. *PLoS Pathog.* 2012; 8:e1002987. [PubMed: 23133376]
98. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, et al. Metal chelation and inhibition of bacterial growth in tissue abscesses. *Science.* 2008; 319:962–965. [PubMed: 18276893]
99. Vitkov L, Klappacher M, Hannig M, Krautgartner WD. Extracellular neutrophil traps in periodontitis. *J Periodontal Res.* 2009; 44:664–672. [PubMed: 19453857]
100. Vitkov L, Klappacher M, Hannig M, Krautgartner WD. Neutrophil fate in gingival crevicular fluid. *Ultrastruct Pathol.* 2010; 34:25–30. [PubMed: 20070150]
101. Cooper PR, Palmer LJ, Chapple IL. Neutrophil extracellular traps as a new paradigm in innate immunity: friend or foe? *Periodontol 2000.* 2013; 63:165–197. [PubMed: 23931060]
102. Palmer LJ, Cooper PR, Ling MR, Wright HJ, Huissoon A, Chapple IL. Hypochlorous acid regulates neutrophil extracellular trap release in humans. *Clin Exp Immunol.* 2012; 167:261–268. [PubMed: 22236002]
103. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog.* 2009; 5:e1000639. [PubMed: 19876394]
104. Behnsen J, Jellbauer S, Wong CP, Edwards RA, George MD, Ouyang W, et al. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. *Immunity.* 2014; 40:262–273. [PubMed: 24508234]
105. Diaz-Ochoa VE, Jellbauer S, Klaus S, Raffatellu M. Transition metal ions at the crossroads of mucosal immunity and microbial pathogenesis. *Front Cell Infect Microbiol.* 2014; 4:2. [PubMed: 24478990]
106. Liu JZ, Jellbauer S, Poe AJ, Ton V, Pesciaroli M, Kehl-Fie TE, et al. Zinc sequestration by the neutrophil protein calprotectin enhances *Salmonella* growth in the inflamed gut. *Cell Host Microbe.* 2012; 11:227–239. [PubMed: 22423963]
107. Zindl CL, Lai JF, Lee YK, Maynard CL, Harbour SN, Ouyang W, et al. IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. *Proc Natl Acad Sci U S A.* 2013; 110:12768–12773. [PubMed: 23781104]
108. de Oliveira KM, da Silva RA, De Rossi A, Fukada SY, Feres M, Nelson-Filho P, et al. Absence of interleukin 22 affects the oral microbiota and the progression of induced periapical lesions in murine teeth. *Int Endod J.* 2015; 48:46–59. [PubMed: 24611911]
109. Deng JS, Qin P, Li XX, Du YH. Association between interleukin-1beta C (3953/4)T polymorphism and chronic periodontitis: evidence from a meta-analysis. *Hum Immunol.* 2013; 74:371–378. [PubMed: 23220433]
110. Li Q, Meng H, Zhang L, Xu L, Chen Z, Shi D, et al. Correlation between single nucleotide polymorphisms in a calprotectin subunit gene and risk of periodontitis in a Chinese population. *Ann Hum Genet.* 2007; 71:312–324. [PubMed: 17069562]

111. Ren XY, Xu L, Meng HX, Zhao HS, Lu RF, Chen ZB, et al. Family-based association analysis of S100A8 genetic polymorphisms with aggressive periodontitis. *J Periodontal Res.* 2009; 44:184–192. [PubMed: 19210342]
112. Sun X, Meng H, Shi D, Xu L, Zhang L, Chen Z, et al. Analysis of plasma calprotectin and polymorphisms of S100A8 in patients with aggressive periodontitis. *J Periodontal Res.* 2011; 46:354–360. [PubMed: 21463326]
113. Nijnik A, Hancock RE. The roles of cathelicidin LL-37 in immune defences and novel clinical applications. *Curr Opin Hematol.* 2009; 16:41–47. [PubMed: 19068548]
114. Currie SM, Findlay EG, McHugh BJ, Mackellar A, Man T, Macmillan D, et al. The human cathelicidin LL-37 has antiviral activity against respiratory syncytial virus. *PLoS One.* 2013; 8:e73659. [PubMed: 24023689]
115. Tripathi S, Tecle T, Verma A, Crouch E, White M, Hartshorn KL. The human cathelicidin LL-37 inhibits influenza A viruses through a mechanism distinct from that of surfactant protein D or defensins. *J Gen Virol.* 2013; 94:40–49. [PubMed: 23052388]
116. Bergman P, Walter-Jallow L, Broliden K, Agerberth B, Soderlund J. The antimicrobial peptide LL-37 inhibits HIV-1 replication. *Curr HIV Res.* 2007; 5:410–415. [PubMed: 17627504]
117. Rapala-Kozik M, Bochenska O, Zawrotniak M, Wolak N, Trebacz G, Gogol M, et al. Inactivation of the antifungal and immunomodulatory properties of human cathelicidin LL-37 by aspartic proteases produced by the pathogenic yeast *Candida albicans*. *Infect Immun.* 2015; 83:2518–2530. [PubMed: 25847962]
118. Tang L, Chen J, Zhou Z, Yu P, Yang Z, Zhong G. *Chlamydia*-secreted protease CPAF degrades host antimicrobial peptides. *Microbes Infect.* 2015; 17:402–408. [PubMed: 25752416]
119. Chromek M, Slamova Z, Bergman P, Kovacs L, Podracka L, Ehren I, et al. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. *Nat Med.* 2006; 12:636–641. [PubMed: 16751768]
120. Danka ES, Hunstad DA. Cathelicidin augments epithelial receptivity and pathogenesis in experimental *Escherichia coli* cystitis. *J Infect Dis.* 2015; 211:1164–1173. [PubMed: 25336727]
121. Singh D, Qi R, Jordan JL, San Mateo L, Kao CC. The human antimicrobial peptide LL-37, but not the mouse ortholog, mCRAMP, can stimulate signaling by poly(I:C) through a FPRL1-dependent pathway. *J Biol Chem.* 2013; 288:8258–8268. [PubMed: 23386607]
122. Putsep K, Carlsson G, Boman HG, Andersson M. Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. *Lancet.* 2002; 360:1144–1149. [PubMed: 12387964]
123. Carlsson G, Wahlin YB, Johansson A, Olsson A, Eriksson T, Claesson R, et al. Periodontal disease in patients from the original Kostmann family with severe congenital neutropenia. *J Periodontol.* 2006; 77:744–751. [PubMed: 16584360]
124. Segat L, Zupin L, Moura RR, Coelho AV, Chagas BS, de Freitas AC, et al. DEFB1 polymorphisms are involved in susceptibility to human papillomavirus infection in Brazilian gynaecological patients. *Mem Inst Oswaldo Cruz.* 2014; 109:918–922. [PubMed: 25410996]
125. Loo WT, Bai LJ, Fan CB, Yue Y, Dou YD, Wang M, et al. Clinical application of human beta-defensin and CD14 gene polymorphism in evaluating the status of chronic inflammation. *J Transl Med.* 2012; 10(Suppl 1):S9. [PubMed: 23046822]
126. Jones EA, Kananurak A, Bevins CL, Hollox EJ, Bakaletz LO. Copy number variation of the beta defensin gene cluster on chromosome 8p influences the bacterial microbiota within the nasopharynx of otitis-prone children. *PLoS One.* 2014; 9:e98269. [PubMed: 24867293]
127. Jaradat SW, Hoder-Przyrembel C, Cubillos S, Krieg N, Lehmann K, Piehler S, et al. Beta-defensin-2 genomic copy number variation and chronic periodontitis. *J Dent Res.* 2013; 92:1035–1040. [PubMed: 24019264]
128. Bentley RW, Pearson J, Gearry RB, Barclay ML, McKinney C, Merriman TR, et al. Association of higher DEFB4 genomic copy number with Crohn's disease. *Am J Gastroenterol.* 2010; 105:354–359. [PubMed: 19809410]
129. Wilson SS, Wiens ME, Smith JG. Antiviral mechanisms of human defensins. *J Mol Biol.* 2013; 425:4965–4980. [PubMed: 24095897]

Figure 1. Cell autonomous autocrine regulation of mucosal epithelial AMPs

IL-1 α released from epithelial cells engages the IL-1 receptor (IL-1R) on the same or proximal epithelial cells establishing an autocrine loop that signals for increased expression of AMPs. Autocrine IL-1 α activates and stimulates DNA binding by the transcription factor C/EBP β which upregulates S100A8/A9. S100A8/A9 and CAMP upregulation may also reflect an NF- κ B-dependent response of epithelial cells to selected microbial pathogens in vitro. When upregulated, S100A8/A9, CAMP and its peptide product LL37 increases antimicrobial activity in the cytoplasm and endosomes of mucosal epithelial cells against invasive bacteria, augmenting cell autonomous immunity without support from inflammatory or immune cells. Surface TLR2 and TLR4 (associated with TLR1 or 6), engage PAMPs and contribute to upregulation of AMPs. Since many pathogens internalize into the endosomes, engagement of intracellular TLRs can orchestrate an innate autonomous immune response.

Figure 2. Convergence of inflammasome signaling in the cell autonomous response

Activation of one of four PRR family members (AIM2, NLRC4 or IPAF, NLRP1, and NLRP3) initiates inflammasome formation. Inflammasomes are organized into sensor proteins such as the NLRs and an adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD-like domain). The sensor proteins discriminate between PAMPs such as peptidoglycan fragments (NLRP1), microbial proteins (NLRC4) and dsDNA from host or microbial sources (AIM2). Inflammasome activators cause simultaneous release of proinflammatory cytokines including IL-1α.